MICRONISATION OF TOBRAMYCIN USING DENSE GAS ANTI-SOLVENT TECHNIQUES

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ABSTRACT

The feasibility of micronising tobramycin by the gas anti-solvent technique was investigated. The micronisation of tobramycin was carried out by precipitating from a solution using dense gas anti-solvent techniques. Two different techniques, the Gas Anti-Solvent (GAS) method and the Aerosol Solvent Extraction System (ASES) were utilised. Tobramycin was dissolved in an organic solvent such as methanol, ethanol and dimethyl sulfoxide. In the GAS process the solution was expanded with CO_2 at 25°C and 35°C with pressures ranging from 50 to 70 bar. In the ASES process the solution was sprayed into a vessel pressurised with carbon dioxide at 25°C and 45°C with the density of 0.017 and 0.018 gmol/cm^3. Sub-micron particles of tobramycin were produced using both techniques. The GAS technique produced uniform micro-spheres that had a low degree of agglomeration. The ASES technique produced smaller particles that were highly agglomerated and in some cases, of irregular morphology. The results of in vitro analysis indicated the fine particle mass (<5 micron) was at least 40%. It is therefore possible to use the drug for dry powder inhalation delivery.

INTRODUCTION

Research in alternative drug delivery methods has intensified in the past few years. One such method is the direct delivery of antibiotics to the respiratory tract. Traditionally a suspension or solution containing the antibiotic compound is delivered into the respiratory system using metered dose inhalers. Preservatives such as phenol are added to the solution to enhance the storage life of the drug. However, these preservatives usually have an unpleasant taste and this fact is further enhanced when the solution is used for aerosol inhalation [1]. The antibiotic can be delivered by inhalation as a dry powder to eliminate the drawback of utilizing preservatives such as phenol. A dry powder inhaler can be used for a drug that possesses a significant mass fraction of fine particles that are less than 5 μm. Particles of between 1-5 μm are transferred to the bronchiolar region while particles of less than 1 μm can reach the alveolar region where the drug is able to cross the thin alveolus membrane into the blood capillaries and subsequently travel into the main bloodstream [2]. Dry powder inhalers have the advantage of not requiring the hand-lung coordination required by pressurised metered dose inhalers (pMDIs), are simple and can overcome the problems of finding a suitable drug formulation for delivery with propellants [3]. In addition, the storage of the antibiotic as a dry powder reduces the degree of degradation at ambient temperature [4].

Tobramycin is an antibiotic from the aminoglycoside group. Aminoglycosides are primarily used to treat infections that are caused by aerobic, Gram-negative bacteria. This group of antibiotics is effective against Pseudomonas, Acinetobacter, and Enterobacter

species [5]. However, aminoglycosides have a serious drawback, as they were very likely to cause nephrotoxicity and ototoxicity as well as neuromuscular paralysis [6]. Large doses of tobramycin are highly toxic and can cause permanent damage to the cells of the cochlea and vestibular system. A high concentration of the antibiotic in the bloodstream can affect the functions of the kidney by causing proximal tubular damage and subsequently, renal failure can occur. Therefore inhalation delivery is able to directly deliver the antibiotic to the site of infection, thus minimising the side effects as well as increasing bioavailability at the area that it is needed.

Traditional micronisation techniques such as spray drying, milling and grinding have been used to micronise pharmaceutical materials. Milling is able to produce fine particles but with a size distribution that is too broad for inhalation delivery. In addition, the shear stress introduced with milling has been shown to affect the chemical stability of the compound [7]. Spray drying requires the use of organic solvents in order to produce the solution that is atomised into a heated drying chamber. Solvents that have a higher boiling point require a higher temperature in order to evaporate. Therefore sensitive pharmaceutical material can undergo degradation during the process. In addition, there is no washing step to remove the residual solvent. Therefore further drying and heating is required to minimise the amount of solvent in the final product. Furthermore, other conventional processes produce fine powder with an electrostatic charge that has poor flow and dispersion properties, resulting in an unsatisfactory dispersion following emission from a dry powder inhaler [4]. Alternative processing methods have been developed in order to facilitate the processing of pharmaceutical materials to suitable forms for aerosol delivery. The Gas Anti-Solvent (GAS) method and the Aerosol Solvent Extraction System (ASES) are two techniques that utilise dense gases as anti-solvents for the precipitation of a drug from solution. These techniques are able to operate at lower temperatures compared to conventional micronisation techniques and residual solvent is removed in the same process by passing dense anti-solvent through the precipitate to produce material that has as low as 20 ppm of residual solvent [8]. Dense gas techniques enable the micronisation of material without the drawbacks of traditional micronisation methods such as the denaturing of sensitive compounds and broad size distributions.

In this study the GAS and ASES techniques was used to precipitate tobramycin in a size range appropriate for dry powder inhalers. It is a significant advantage if tobramycin can be delivered directly to the site of infection. In the case of treating respiratory tract infections, it is beneficial to administer the antibiotic through inhalation, as it would minimise the amount of tobramycin that absorbs into the bloodstream. By delivering the antibiotic in a dry powder form, problems with storage and convenience are also largely eliminated. Therefore the method to achieve this is by dry powder inhalation.

EXPERIMENTAL

***Material*:** The material used was tobramycin base (98.5%) that was provided by Biogal Pharmaceutical Works, Hungary. Methanol (HPLC grade 99.7%), ethanol (HPLC grade 99.7%) and dimethyl sulfoxide (DMSO) (HPLC grade 99.9%) were purchased from Sigma. Industrial grade liquid carbon dioxide (99.95%) from BOC gases was used as the anti-solvent in GAS and ASES. The analytical reagent o-phthaldialdehyde was purchased from Sigma.

Procedure: The experimental procedures for the GAS and ASES processes were described previously by Warwick *et al*. [9]. In summary, for the GAS process a solution containing tobramycin at a certain concentration was prepared and injected into a high-pressure vessel (Jerguson Sight Gauge, Series 32). At the bottom of the vessel, a frit was placed to sparge

carbon dioxide into the solution and facilitate the mixing and collect the precipitated powder. Carbon dioxide was gradually added to the solution using a syringe pump. After the system was expanded, the drug precipitated and the solvent was then purged from the system by adding CO_2 at constant pressure to the vessel. The residual solvent was removed by washing the precipitate with at least four volumes of the pressure vessel. The system was then depressurized and the powder was collected for characterization.

The experiments using ASES were carried out by spraying the solution through a coaxial stainless steel nozzle. The inner nozzle of 0.25 mm inner diameter was used to introduce the drug solution while the larger outer nozzle of 2.5 mm inner diameter was used to introduce the carbon dioxide. The system was first pressurised with carbon dioxide and left to equilibrate at the desired temperature. A needle valve was used to adjust carbon dioxide flowrate at 10-12 mL/min. The drug solution was then introduced by a HPLC pump (Waters Model 510) at a flowrate of 0.2 mL/min. After the desired amount of precipitate was collected, the precipitate was washed with CO_2 at the operating pressure to remove residual solvent. At least 300 mL CO_2 was used to wash the sample.

Scanning electron microscopy was performed to study the morphology of the precipitate. A small amount of sample was placed on a piece of carbon tape and affixed to an aluminium stub. This sample was then gold coated and the SEM analysis was performed with a Hitachi S4500 at 5 kV voltage.

Aerosol performance of the processed powder was measure in vitro using an 8-stage stainless steel Anderson cascade impactor (Copley Scientific). The collection plates were coated with a 50/50 mixture of propylene glycol and methanol, and filter paper (Type GF-50) was used for the final stage to collect particles that were less than 0.26 μm in size. A gelatin size 3 capsule (Parke-Davis) loaded with 20 mg of tobramycin was dispersed using an Aerolizer Inhaler (Novartis) with a flowrate of 60 L/min. The powder captured by each stage was collected by washing with 10 mL of distilled water. The solution was then diluted 10 times before UV analysis. An o-phthaldialdehyde spectrophotometric method was used because tobramycin does not have an absorbance peak in the UV wavelength [10]. The UV absorbance of the derivatives of tobramycin at 333nm was measured using a Hewlett Packard UV-Vis spectrophotometer.

The polymorphic form of tobramycin was analysed by differential scanning calorimetry (DSC) using Thermal Analysis equipment. The analysis was carried out in the temperature range of 20 to 300ºC at a rate of 10ºC/min.

RESULTS AND DISCUSSION

The effects of variables such as temperature, solvent, concentration, and pressurisation rate on the particle characteristics of tobramycin precipitated by the GAS technique were determined. Methanol, ethanol and DMSO were used as tobramycin exhibited reasonable solubility in these solvents and all can be expanded by carbon dioxide [11]. The concentration was varied between 0.1 and 0.2 wt%, and temperatures between 25 and 35ºC were studied. Unprocessed tobramycin was in the form of irregular shape particles with a particle size in the range of 0.1 to 3 μm. The products from the GAS process included uniform submicron spherical particles.

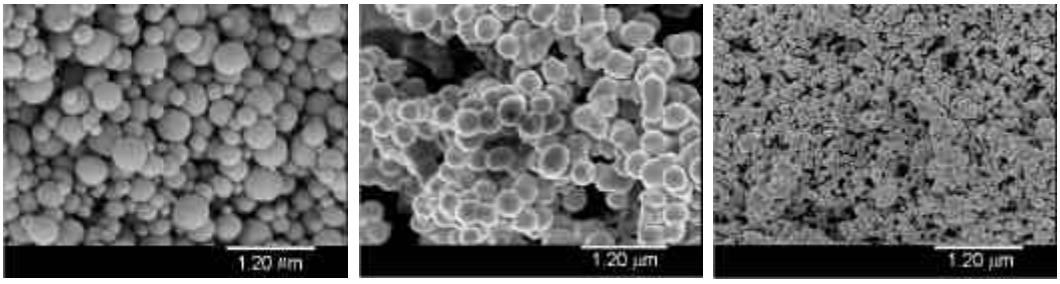
The effect of pressurization rate on the particle size of tobramycin was studied by expanding a 0.1 wt% solution of tobramycin in methanol with CO_2 at a rate of 3-4 bar/min. Uniform spherical particles with sizes of 0.1 to 0.5 μm were produced as shown in Figure 1(a). To observe the effect of the rate of pressurisation, the experiment was repeated at a pressurisation rate of around 12 bar/min. The faster pressurisation rate also produced uniform particles that

were of the same diameter as the particles from the 3-4 bar/min condition. The effect of instantaneous pressurisation was also observed in the form of tightly packed and highly agglomerated particles.

Changing the solvent changes the environment in which precipitation occurs and may possibly even change the precipitation mechanism. To observe the effect of a different solvent, dimethylsulphoxide was used instead while keeping all other parameters identical to the conditions of Figure 1(a). As shown in Figure 1(b), highly uniform 0.4 μm particles were produced. Even though additional volumes of carbon dioxide were used for washing, the particles were coated with a layer of residual DMSO. The solubility of tobramycin in ethanol was much lower than both methanol and DMSO. Therefore a solution of only 0.03wt% was precipitated while maintaining the other conditions. The combination of ethanol and lower concentration produced slightly agglomerated 0.12 μm particles.

The effect of temperature on the particle size of tobramycin precipitated by the GAS was observed using a 0.1 wt% solution in methanol and 3-4 bar/min pressurisation rate while increasing the temperature to 35ºC. The increase in temperature from 25 to 35°C resulted in an increase in the particle size distribution from 0.1-0.5 μm to 0.1-0.8 μm. The increase may be due to an increase in growth rate [12].

Various concentrations of tobramycin in methanol were prepared and the solution was expanded at 25°C. It was found that by lowering the concentration of the tobramycin from 0.1 wt% to 0.05 wt%, the particle size decreased slightly and there was more uniformity in size distribution. By increasing the concentration to 0.2 wt% and keeping the other parameters constant, the particle size increased slightly but there was also an increase in the number of smaller particles, thus broadening the size distribution.



(a) (b) (c)

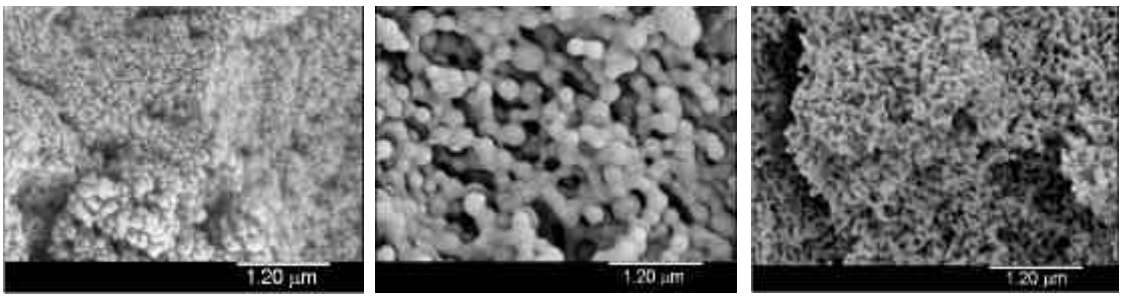
Figure 1:Tobramycin precipitated at 25°C and 3-4bar/min using the GAS method. (a) methanol 0.1wt%, (b) DMSO 0.1wt% and (c) ethanol 0.03wt%

The micronisation of tobramycin was also carried out using the ASES method. The concentration was varied between 0.2 and 1.0 wt%, the temperature between 25 and 45ºC and the carbon dioxide density between 0.017 and 0.018 mol/cm^3. By spraying a 0.2 wt% solution in methanol at 25°C and 86.5 bar (0.018 mol/cm^3), small particles in the range of 100 to 200 nm were produced. The particles produced by the ASES technique were smaller than the ones formed by the GAS process. The degree of agglomeration between the tobramycin particles produced by the ASES technique was increased when the temperature of the process was increased from 25°C (subcritical) to 45°C (supercritical). By changing to supercritical conditions, solvent and anti-solvent are more miscible and thus there is a rapid decrease in surface tension, producing a gaseous jet instead of small droplets [9].

The effect of concentration was observed by increasing the concentration to 1.0 wt% from 0.2 wt% while keeping the other parameters constant. Larger particles that were joined together were formed. The increase in concentration resulted in a more viscous solution,

which tends to delay atomisation. In addition, supersaturation is achieved more rapidly with higher concentrations, thus causing the particles to form before atomisation is able to occur [9].

Carbon dioxide density can influence the particle size and morphology. In order to study the effect, the temperature and pressure were changed from 25ºC and 86.5 bar to 35ºC and 112.1 bar in order to lower the carbon dioxide density from 0.018 to 0.017 mol/cm^3. The particles that were formed tend to coalesce together in aggregates as shown in Figure 1(c). The decrease in the anti-solvent density reduces its solvent power, thus increasing the interaction between methanol and tobramycin, thereby explaining the increase in the degree of coalescence [13].



(a) (b) (c)

Figure 2: Tobramycin precipitated using the ASES method. (a) 0.2wt%, 25^{o}C and 86.5 bar, (b) increased concentration of 1.0wt%, (c) lower carbon dioxide density of 0.017mol/cm^3 by using conditions of 35^{o}C and 112.1 bar.

The micronised tobramycin in Figure 1(a) that was produced by the GAS method was then used to carry out a study on aerosol performance. The micronised material produced agglomerated. Analysis using the cascade impactor indicated that at least 40% of the fine particle mass was suitable for use in dry powder inhalation delivery.

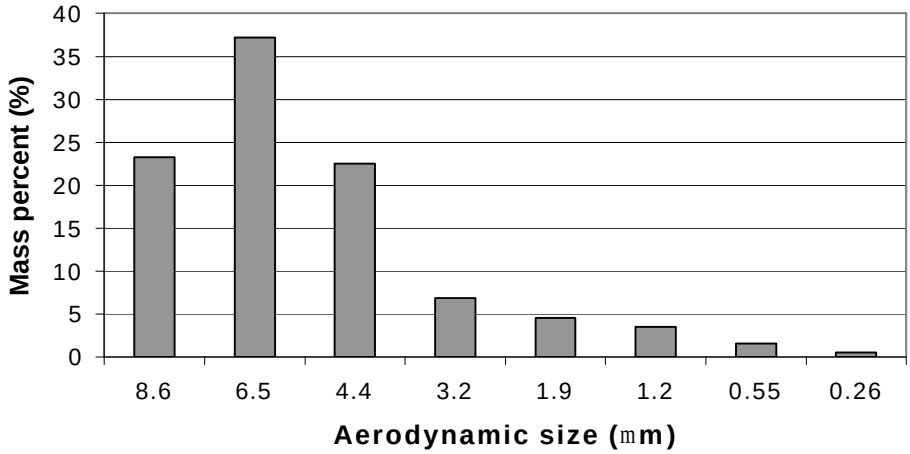


Figure 3: Aerosol performance of tobramycin micronised using GAS at 25ºC, 3-4 bar/min, 0.1wt% methanol.

Analysis by differential scanning calorimetry (DSC) showed that the unprocessed material was crystalline with a melting peak of 230ºC as confirmed by literature. During the GAS micronisation process, tobramycin was precipitated as an amorphous powder, which was missing the melting peak in the thermogram as shown in Figure 4.

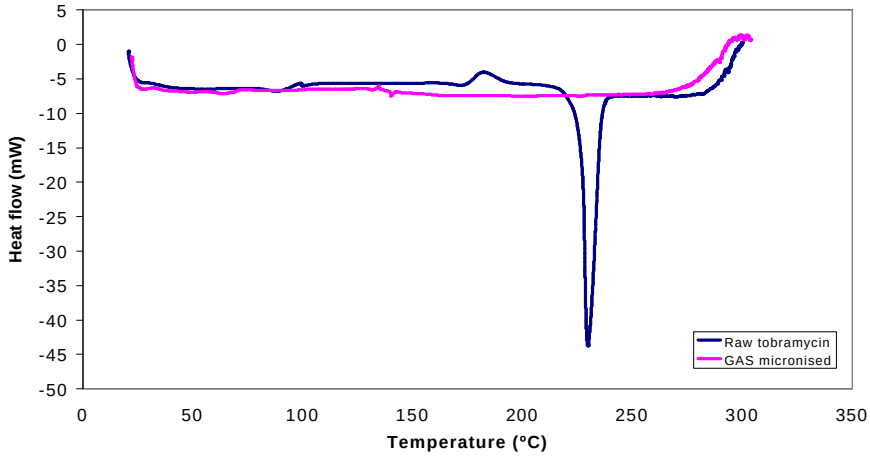


Figure 4: DSC analysis of the unprocessed and GAS micronised tobramycin. The conditions were 25ºC, 3-4 bar/min and 0.1wt% methanol

CONCLUSION

Tobramycin was successfully micronised using dense gas anti-solvent techniques. Both the ASES and GAS methods produced fine particles, but with different degrees of agglomeration. The particles produced using ASES were smaller but had a higher degree of agglomeration. The tobramycin particles produced by GAS were more uniform and of a regular spherical morphology. A comparison of the three solvents used in this study showed that methanol was the most suitable for use in the micronisation of tobramycin. Analysis of the GAS micronised material showed that it was suitable for dry powder inhalation delivery.

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